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Physiological Changes in Adult Rats Exposed to an ELF Rotating Magnetic Field

by
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Several experimenters (Altmann, 1969; Ludwig and Mecke, 1968; Persinger et al., 1971) have reported behavioral modifications following exposure to ELF (extreme low frequency) electromagnetic fields. A more precise description of the sources and characteristics of these fields is reported elsewhere (Ossenkopp, Koltek and Persinger, 1972).

Physiological changes have also been noted following ELF-EM exposure. Exposure of hamsters (Altmann, 1969) or mouse liver tissue (Lotmar, Ranscht-Froesmdorff and Weise, 1969) to such fields is associated with changes in oxygen uptake and respiration rate. In an unpublished study, Persinger (1967) noted that adult rats exposed to a 0.5 Hz, 3-30 gauss rotating magnetic field (RMF) consumed significantly more (13%) water than control rats. These reports in conjunction with the observation that rats exposed during their prenatal development to an ELF (0.5-3 gauss) RMF had significantly heavier thyroid and testicle weights, suggests that measurable changes in the gross physiology of adult rats exposed to such fields during varying periods of time could occur. The greater open field activity, aggressive behavior, water consumption and changes in food consumption noted in the adult RMF-exposed rats (Persinger, 1967), behaviors characteristic of increased thyroid activity (relative "hyperthyroidism"), indeed suggest that this structure may show measurable changes in gross weight following ELF-RMF exposure.

METHOD

SUBJECTS. In four experiments (Experiments I, II, III and IV), 44 (12, 12, 12 and 8 rats respectively) Holtzman strain male rats 120, 150, 80 and 115 days of age at the beginning of the experiments were used as subjects. The rats used in Experiment II had been used in a maze study between 80 and 100 days of age, while in the other three experiments, the rats were naive.

APPARATUS. Two rotating magnetic field (RMF) apparatus were used. Essentially, the RMFs were created by two horseshoe magnets rotating in opposite directions about their major axes at 30 rotations per minute. The magnets in apparatus A, a diagram of which has been reported earlier (Persinger and Pear, 1972), were rotated by an electric motor that drove a pulley-shaft system to which the magnets were attached. In apparatus B, each magnet was rotated by a separate variable speed transmission (Persinger et al., 1972). The major axes of the magnets were alligned in a N-S direction in Winnipeg, Canada, and rotated in either a clockwise (apparatus A) or counterclockwise (apparatus B) direction with respect to magnetic north. The RMF of apparatus A varied from 3-10 gauss

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(as measured by a Rawson-Lush Gaussmeter) in the central exposure compartment, nearest the magnets, and 0.5-3 gauss in the two peripheral exposure compartments. The RMF of apparatus B varied from 5-30 gauss in the central exposure compartment, and 1-5 gauss in the two peripheral compartments.

PROCEDURE. Rats were weighed and placed into one of two 70 x 23 x 26 cm rubber tile cages in the experimental rooms so that the average rat weight in one cage did not differ by more than three grams from the average rat weight in the other cage. The cages were divided into 3 compartments of similar volume, and covered with 2.5 cm wire mesh. In Experiments I-III, two rats were placed in each compartment, while in Experiment IV (which involved only 8 subjects), only two compartments of each cage were used (still 2 rats per compartment). One cage was then placed in either RMF-apparatus A (Experiments I and IV) or apparatus B (Experiments II and III). Since the motor in RMF-apparatus A was secured to the plywood base that produced some vibration, the cage was placed on a platform 1.3 cm above and independent of the base. The cage placed in RMF-apparatus B also did not show any measurable vibration from the apparatus. The other cage was placed in the control area 3 m from the nearest magnet. Although a Rawson-Lush gaussmeter showed no deviation at this distance, a lab compass (8 cm needle) showed a continuous pulsing 0.2° change in declination while the RMFs were operating. The rats were exposed to the experimental (RMF and control) conditions in Experiments I, II, III and IV for 10 days (18-28 November 1970, 5 days (20-25 December 1970, 21 days (6-27 March 1971) and 26 days (12 April-8 May 1971).

Cedar shavings were used as absorbent material in the cages and were removed once every two to three days, during which time the animals were removed from the experimental conditions, weighed, and given fresh food. The amount of food consumed was recorded only for Experiment IV. During cleaning of cages, the rats exposed to the RMF were out of the field for about 5 min. Illumination was constant and provided by a 20-w fluorescent lamp 45 cm above each cage. Cage temperature averaged $24 \pm 0.5^{\circ}$C in Experiments I, III, and IV, and $20 \pm 1^{\circ}$C in Experiment II. Temperature between the RMF and control cages did not vary by more than 0.5°C at any given time. Background sound pressure level (as measured by a B and K Precision Sound Level Meter) averaged 67 ± 3 db in the exposure area of RMF-apparatus A (from the motor) and 65 ± 4 db in the control exposure area. The background sound pressure level in the exposure area of RMF apparatus B averaged 77 ± 3 db, with 73 ± 2 db in the control area. As a comparison, the background sound pressure level in the department's main colony room was 62 ± 2 db. Other physical variables were not recorded. Animals received Purina food and water ad libitum. The amount of water consumed each day by each group was measured with a graduated (in ml) cylinder. The amount of food consumed was derived by recording the weight of food pieces remaining when the exposure cages were cleaned.

At 130, 155, 100 and 141 days of age, after 10, 5, 21, and 26 days of exposure to experimental conditions, the rats in Experiments I-IV, respectively, were weighed and then killed by decapitation. The time between removal from the experimental conditions and decapitation averaged 3 minutes. Wet weights of adrenals, thyroids, and testicles (except in Experiment II) were measured on a Sartorius balance. Any gross morphological changes were noted. In Experiments I, III, and IV, blood, taken from the collected pool following decapitation, was drawn into a blood diluting pipette to determine circulating blood eosinophil counts. The sample was treated as outlined by Miale (1967) while the counting procedure outlined by Wintrobe (1956) was followed using the Fuchs-Rosenthal counting chamber.

RESULTS

The average per cent of increase or decrease of water consumption for every two days of exposure for RMF-exposed and control groups in the four experiments is presented in Fig. 1.

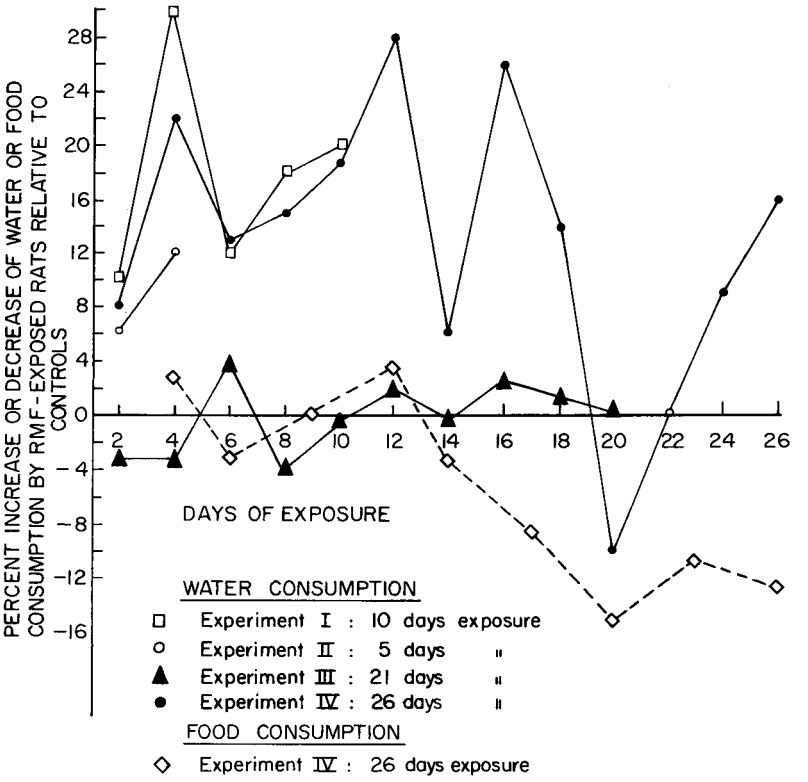

Fig. 1. Per cent increase (or decrease) in water consumption of RMF-exposed rats relative to controls in Experiments I (□), II (o), III (▲) and IV (●) or in food consumption in Experiment IV (◇) as a function of exposure days. In Experiments I-IV, the rats were 120, 150, 80 and 115 days of age, respectively, at the beginning of experiments.

The RMF-exposed groups in Experiments I, II and IV averaged 18%, 9%, and 13% more water consumption than their respective control groups. The differences in Experiments I and IV were significant beyond the 0.01 level. The RMF-exposed rats in Experiment III averaged 1% less water than controls. Although not plotted on the figure, there was a consistent tendency for RMF-exposed rats in the low intensity 0.5-3 gauss compartments to consume more water than rats in the central compartments. This tendency was not observed with control rats. Food consumption, which was taken only in Experiment IV, is also shown in

Fig. 1. There was a consistent, but statistically non significant tendency for RMF-exposed rats to consume less food as the duration of exposure increased.

The average, median and SD (standard deviations) values for thyroid weight (μ g thyroid/g body weight), total testicle weight (mg testicle/g body weight) and body weight again (g) for RMF-exposed and control groups are presented in Table 1.

TABLE 1. Means ($\bar{X}$), medians (M^{dn}) and standard deviations (SD) for relative thyroid weight, testicle weight and body weight gain by rats in RMF and control (C)

	EXPERIMENT I 10 days exposure 120-130 days old		EXPERIMENT II 5 days exposure 150-155 days old		EXPERIMENT III 21 days exposure 80-100 days old		EXPERIMENT IV 26 days exposure 115-141 days old	
	Thyroid (μg/g body weight)							
	RMF	C	RMF	C	RMF	C	RMF	C
$\bar{X}$	56.0	87.7	77.8	82.2	282.3	271.5	171.0	144.4
M^{dn}	63.0	87.5	72.0	84.5	273.0	280.5	140.5	147.0
SD	19.1	18.1	60.4	16.4	104.6	80.3	95.1	15.2
n	6	6	6	6	6	6	4	4
t = 2.94**								
	Testicles (mg/g body weight)							
$\bar{X}$	8.87	8.62			9.26	9.26	8.09	7.35
M^{dn}	9.16	8.78			9.33	9.51	8.00	7.30
SD	0.28	0.23			0.11	1.01	0.13	0.11
n	6	6			6	6	4	4
t = 1.68						1		2.72**
	Body weight gain (g/day)							
$\bar{X}$	5.0	1.5	3.5	2.2	2.9	2.8	1.9	1.8
M^{dn}	5.1	1.4	3.2	2.3	2.9	2.5	2.0	1.9
SD	1.4	0.7	1.5	1.1	0.4	0.6	0.3	0.6
n	6	6	6	6	6	6	4	4
t = 5.79***								

*) $p < 0.05$.
**) $p < 0.02$.
***) $p < 0.001$.

In Experiment I (10 days of exposure) the RMF-exposed 56.0 μg/g body weight of thyroid tissue while the controls averaged 87.7 μg/g. This difference was found by t-test to be significant beyond the 0.02 level (t = 2.94; two tailed). In Experiments II, III, IV, the RMF-exposed rats averaged 77.8, 282.3 and 171.0 μg/g body weight while the controls average 82.2, 271.5, and 144.4 μg/g body weight. These differences were not statistically significant. The average total testicle weight for RMF-exposed rats in Experiments I, III and IV was 8.87, 9.26 and 8.09 mg/g body weight while the controls average 8.62, 9.26 and 7.35 mg/g body weight. Only the differences in Experiment IV were significant beyond the 0.05 level (t = 2.72). It was also noticed that RMF-exposed rats in Experiments I and IV consistently exhibited greater volumes of semen following decapitation relative to controls. No numerical measures were taken.

From the time of initial exposure to decapitation, RMF-exposed rats in the four experiments averaged 50.3, 17.3, 61.3 and 49.2 g weight gains while the controls averaged 14.6, 10.8, 58.8 and 47.0 g weight gains. These values represented daily weight gains of 5.0, 3.5, 2.9 and 1.9 g/day for the RMF-exposed groups and 1.5, 2.2, 2.8 and 1.8 g/day for the control groups, respectively. The difference in Experiment I was significant beyond the 0.01 level (t = 5.79). The average circulating blood eosinophil counts and relative adrenal weight for RMF groups in Experiments I and IV was 20.5 and 11.3 counts, and 48.3 and 46.6 μg/g body weight of adrenal tissue while the controls averaged 28.7, 21.5 counts and 48.9, 50.5 μgm/g of adrenal tissue. Although there was a trend for RMF-exposed rats in Experiment IV to show eosinopenia and reduced adrenal weight, the t values for these measures (t = 1.81, 1.95) were not significant.

DISCUSSION

The results of Experiments I, II, and IV, indicate that prolonged exposure to a 0.5 Hz, 0.5-3 or 3-30 gauss RMF is associated with progressive changes in body weight, thyroid weight, testicle weight, water consumption, adrenal weight, and circulating blood eosinophil counts, although the latter two measures were not statistically significant in any of the experiments. Rats that had been exposed to the RMF for 26 days showed relative eosinopenia and significantly increased testicle weights, while those exposed only 10 days showed a non-significant trend in that direction. Similarly, relative thyroid weights of the RMF-exposed rats were significantly less after 10 days of exposure, although the trend was evident after five days of exposure. The relatively greater variance of thyroid weight in the RMF-exposed groups was alse noted in the 26 day exposure rats, although these animals did not differ significantly from controls in average relative thyroid weight. The variables controlling the latter changes are not clear.

As noted in the unpublished data (Persinger, 1967), RMF-exposed rats in Experiments I, II, and IV also consumed significantly more water than controls. Together these experiments indicate that water consumption progressively increased up to exposure day 12, after which (at least in Experiment IV), the difference began to oscillate. Although the controlling variables are not clear, the decrease in water consumption beginning around exposure day 14, might have been associated with some environmental change which was in turn responsible for the lack of significant difference in thyroid weight between RMF-exposed and control rats killed on exposure day 26. It is also interesting that the RMF-exposed rats began to eat less food after about 14-17 days of exposure, until by days 20-26 they were averaging 13% less food consumption than controls. This decrease in food consumption of the RMF-exposed rats in Experiment IV may account for the lack of significant weight gain that had been observed in the RMF-exposed rats in Experiment I. Similar decrements in weight gain and even weight loss, relative to controls, has been observed in rats exposed longer than 30 days

to the RMF (Persinger, 1967). The possibility of interacting effects from ambient ELF waves (which were not shielded out in this study) associated with environmental disturbances is also being investigated.

Collectively, the significant increase in body weight and water consumption in this study, and the increased aggressive, open field, and non-criterion (error) maze behavior emitted during previous studies (Persinger, 1967; Persinger et al., 1972), taken in conjunction with the decrease in relative thyroid weight, could suggest that the ELF-exposed rats were more "hyperthyroid". The results of other experiments indirectly supports this possibility. Frey and Goeke (1969) observed a 4-7% increase of radioactive uptake in the thyroid (indicative of increased thyroid activity) during "biologically unfavorable" (labile) weather conditions. Lotmar and Ranscht-Froemsdorff (1968) observed that under similar labile conditions, respiration rate in tissue was markedly decreased. Postulating that this decrease in respiration rate was associated with increased incidence of ELF-EM fields during the labile weather conditions, Lotmar, Ranscht-Froesmdorff and Weise (1969) exposed liver tissue in the laboratory to artificial fields simulating natural forms and intensities. A similar decrease in respiration rate was observed. Hence the increase in ^{131}I uptake noted by Frey and Goeke (1969) may have been a product of the increased incidence of ELF fields associated with the labile weather.

Although further tests must be made (e.g., circulating T_3-T_4 levels), the possibility that increased thyroid activity follows application of ELF-EM fields could explain the "hypothyroid-like" behavior, e.g., retardation of teeth eruption, etc.) displayed by rats exposed during their prenatal development to the RMF (Ossenkopp, 1971; Ossenkopp, Koltek and Persinger, 1971).

Since some thyroid compounds can cross the placenta (Hommes, Wilmink and Richters, 1969), an increase of thyroid chemicals in the pregnant adult female exposed to the RMF would produce a net increase in the level of such compounds in the developing fetus. At birth, when the neonate is removed from this increased thyroid chemical environment, a net relative "hypothyrodism" would result. In this context it is interesting that Brezowsky and Ranscht-Froemsdorff (1966) have noted a correlation between decrease ELF wave occurrences and increase in frequency of myocardial infarctions (MI). Recent work by Felt (1969) has shown that the hormonal signs of hypothyroidism such as lower values of bound thyroxine, are noted in MI patients.

The results of Experiment III may suggest that age at the time of exposure is a critical variable. In this experiment, RMF-exposed rats did not show the significant water consumption or tissue weight changes noted in the other three experiments, despite 21 days of exposure. If indeed RMF-exposure produces a slight increase in thyroid activity, such effects would be masked by the already active thyroid of the young rat. However, as the rat matures, and the thyroid decreased in activity, RMF-exposure could, then induce noticeable changes relative to controls. However, further data must be collected before statements concerning a possible age effect can be made.

One mechanism by which ELF-EM field may initiate correlative changes in physiology and behavior is by altering the characteristics of the colloid contained in the thyroid follicles of the exposed rats. Piccardi (1962) and others have noted that the precipitation rates of organic (blood) and inorganic colloids in water solution can be affected by VLF fields. Furthermore, Stewart (1959, 1966, 1969) has shown that many biological compounds, such as lipids, present in the adrenal cortex, ovaries, myelin and artheromatous arteries exist at body temperature in a characteristic mesophase (liquid crystalline state). If the thyroid colloid molecules exhibited paracrystalline behavior, ELF-EM absorption might also be possible. Ludwig (1968), supported by Meirer and Saupe's (1966) data, states

that the Debye-absorption frequencies in liquid crystals are, owing to their great intermolecular forces, in the same range of at least VLF signals. Static magnetic fields (Sackmann, Meiboom and Snyder, 1967) static electrical fields (Heilmeier, Zanoni and Goldmacher, 1969) and ELF fields (Muller, 1966), with intensities much higher than those which occur in nature, can convert a cholesteric mesophase into a nematic one that results in different characteristics in the incident light reflected. Perhaps more subtle influences of natural ELF-EM fields upon biological liquid crystals are possible.

Ludwig (1971), considering data collected by Persinger (1969) on rats exposed prenatally to ELF-EM fields, has derived a mathematical theory to show that low intensity ELF fields can affect physiological processes. According to his calculations, the ELF fields influence the calcium ion movements in such a fashion as to increase the permeability of cell membrane crystal fluid structures and result in increased action potentials. He further states that the ELF-effect is greatest where the synapse density is highest and where the summation effect of action potentials is likely to take place. As a result, Ludwig suggests that the primary effect be sought in the hypophyseal regulatory center in the CNS and that changes in the endocrine system are secondary. With adult exposures this explanation seems quite plausible since similar changes in physiology and behavior have been noted following lesions or stimulation of the pituitary. In the rat, the portal system does not begin to function until about five days of age. Hence, Ludwig's theory would suggest that the effect of prenatal ELF exposure would primarily be upon the developing pituitary structures, where activity at maturity might consequently be modified. Since Carpenter (1959) has indicated that release of thyrotropic hormone from the fetal pituitary begins on the 19th day of fetal development, changes in pituitary and consequent thyroid function due to prenatal ELF-EM exposure, may be possible.

The magnetic component of the field used in this study varied from 0.5 to 10 gauss, which is higher than natural intensities. The electrical component of the field, on the other hand, has been calculated to be about 10^{-5}V/m, well within natural intensity ranges. Ludwig (1968) has conjectured theoretically that amplitude should be of minor importance, frequency being the more critical variable. Lotmar, Ranscht-Froesmdorff and Weise (1969) have noted that the separate application of a "bad" weather H-field only did not produce the usual decrements of respiration rate associated with E - H-field program applications. (It is also interesting that in our experiments, rats in the less intense ELF-RMF areas (0.5-3 gauss) consumed more water than those in the more intense areas.)

In conclusion the results of these experiments suggest that the effects of exposure of rats of a given age to an ELF-RMF is progressive and a function of total duration of exposure. However, it must be emphasized that although the differences between RMF-exposed and control rats were statistically significant in some experiments, the magnitude of such differences was not consistent. In order to determine more specifically the precise controlling stimuli and physiological mechanisms involved with ELF-EM exposure, systematic replications of this research are in progress.

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ABSTRACT.- In three Experiments (I, II, IV), adult male rats, between 115 and 150 days of age were exposed to either a 0.5-3 or 3-30 gauss ELF (0.5 Hz) rotating magnetic field (RMF), for 5, 10, or 26 days. The rats exposed to the RMF for 10 and 26 days averaged significantly ($p < 0,05$) greater water consumption than controls. The group exposed to the RMF for 5 days also consumed more water, although the difference was not significant. ELF-RMF-exposed rats also showed a progressive decrease ($p < 0.02$) in relative thyroid weights, but increase in body weight gain ($p < 0.001$) up to 10 days of exposure, and increase in testicle weights ($p < 0.05$) up to 26 days of exposure. No significant differences were found between groups for circulating blood eosinophil counts or relative adrenal weights, although again the differences did increase with duration of exposure. In a fourth Experiment (III), rats that were 80 days of age at the beginning of 21 days of exposure did not show any significant differences in the above measures from the control group. The changes in behavior and physiology associated with ELF exposure is discussed in terms of its effects upon the thyroid and its probable liquid crystalline properties.

ZUSAMMENFASSUNG.- In drei Untersuchungen (I, II, IV) wurden männliche, 115-150 Tage alte Ratten entweder einem 0,5-3 oder einem 3-30 Gauss ELF (0,5 Hz) rotierenden magnetischen Feld (RMF) für 5, 10 oder 26 Tage ausgesetzt. Die Ratten, die dem RMF für 10 und 26 Tage ausgesetzt waren, hatten einen signifikant ($p < 0,05$) höheren Wasserkonsum als die Kontrollgruppe. Auch die Tiere, die dem RMF für 5 Tage ausgesetzt waren, tranken mehr Wasser ($p > 0,05$). Die exponierten Tiere zeigten auch eine fortschreitende Abnahme ($p < 0,02$) des relativen Schilddrüsengewichts, dagegen eine grössere Gewichtszunahme ($p < 0,001$) bei bis zu 10 Tagen Exponierung und Zunahme des relativen Hodengewichts ($p < 0,05$) bei bis zu 26 Tagen Exponierung. Die Unterschiede in der Zahl der zirkulierenden Esinophilen und im relativen Nebennierengewicht waren nicht signifikant. In der vierten Untersuchung (III) mit Ratten die bei Versuchsbeginn 80 Tage alt waren fanden sich nach 21 Tagen Exponierung keine Unterschiede beim Vergleich mit der Kontrollgruppe. Die physiologischen Veränderungen durch ELF-Exponierung, werden über eine Wirkung auf der Schilddrüse und ihre möglichen flüssigen und kristallinen Eigenschaften erklärt.

RESUME.- Lors de trois essais (I, II et IV), on a exposé des rats mâles, adultes, âgés de 115 à 150 jours à un champ magnétique tournant (CMT) d'une très basse fréquence (FTB) - de 0,5 Hz - soit de 0,5 à 3 Gauss, soit de 3 à 30 gauss, et cela durant 5, 10 ou 26 jours. Les rats exposés au CMT durant 10 ou 26 jours ont bu plus d'eau que des rats de contrôle et cela de façon significative ($p < 0,05$). Le groupe exposé au CMT durant 5 jours seulement a aussi bu davantage d'eau, mais la différence n'est alors pas significative. Les rats exposés à la fois au CMT et au FTB ont aussi montré une diminution significative ($p < 0,02$) du poids relatif de la glande thyroïde, une augmentation du poids du corps ($p < 0,001$) jusqu'au 10ème jour d'exposition et une augmentation du poids des testicules pour 26 jours d'exposition. On n'a par contre pas constaté de différences significatives entre les groupes quant au nombre d'oesinophiles circulant dans le sang, ni quant au poids des glandes surrénales, bien que ces différences augmentassent avec la durée d'exposition. Dans un quatrième essai (III), des rats âgés de 80 jours au début d'une période d'exposition de 21 jours n'ont présenté aucune différence quant aux paramètres mentionnés ci-dessus. Les changements du comportement et de la physiologie dûs à une exposition au FTB sont discutés par rapport aux répercussions d'une telle exposition sur la thyroïde et ses propriétés liquides et cristalline.